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Phenotypic screening for identification of new sources of resistance against brown Planthopper [*Nilaparvata lugens* (Stal.)] in Rice

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Abstract
Host plant resistance is an effective and environmentally friendly approach to manage brown planthopper. Though breeding for resistance to BPH was initiated earlier, identification of new resistant sources for BPH is still in progress. Screening for resistance to BPH results in identification of new resistant sources. Use of mutagenesis in breeding has involved forward genetic screens and selection of mutants with improved traits and their incorporation in breeding programmes. Hence studies were undertaken to screen a total of 432 Ethyl Methane Sulphonate (EMS) induced mutants were phenotyped using protray screening test (PST). Among the different rice mutants N22-CC-DTM-893 was considered as resistant and three other mutants N22-MG-145, N22-MG-491 and N22-MG-516 considered as moderate resistant. To study their mechanism of resistance, resistant mutants along with N22 were evaluated for the different parameters of antibiosis and tolerance experiments. These results helped in relative quantification of BPH resistance levels in the mutants. So N22-CC-DTM-893 is considered a new effective source of BPH resistance and could be used as new donors and utilized in resistance breeding programmes in rice.

Keywords: Brown planthopper, EMS induced mutants, Protray screening test, resistance breeding, Rice

Introduction
Rice (*Oryza sativa* L.) is one of the main staple food for one-third of the world's population. There are many constraints in rice production and among them insect pests remain a primary problem in all rice-growing areas (Narayanasamy *et al.*, 2014) [21]. Brown planthopper (BPH) *Nilaparvata lugens* L. is one of the important pests of rice causing huge yield losses every year in rice throughout tropical, subtropical and temperate regions in Asia (Park *et al.*, 2008) [25]. During plant growth development from seedling to reproductive stage, BPH sucks the phloem sap, causing whole plant senescence called hopper burn (Dale 1994) [6]. BPH losses in grain yield range from 10% in moderately affected fields to 70% in those severely affected fields and the damage to the standing crop sometimes reached 100%. In addition to this BPH also transmit viral diseases such as ragged stunt virus and grassy stunt virus diseases to rice plants (Jena *et al.*, 2006) [13]. Outbreaks of BPH are very frequent in tropical Asia and have caused heavy yield losses frequently (Normile, 2008) [22].

Many chemical insecticides have been recommended for the control of planthoppers. However, extensive application of insecticides may affect behavioural, physiological and biochemical features of insects leading to the development of insecticide resistance in hoppers (Matsumura *et al.*, 2009) [18] and rapid evolution of pesticide-tolerant biotypes of insect pests. These chemicals also have detrimental impact on natural enemies (Balakrishna and Satyanarayana, 2013) [3]. Hence, development of insect-resistant rice varieties is considered a viable and ecologically sustainable approach for controlling this devastating insect pest (Chen *et al.*, 2011) [5]. Natural variation in rice plants to BPH resistance is limited, as rice is an obligate self-pollinating crop. Chemical and physical mutagenesis has been used to induce mutations and create novel variations in rice genotypes, which are then used as sources for the development of new resistant genotypes (Wu *et al.*, 2005) [34]. The novel genetic variation obtained from either spontaneous or induced mutation can be exploited in crop genetics and it can be applied in functional genomics and molecular breeding (Jiang and Ramachandran, 2010) [15]. Chemical mutagens like ethyl methane sulphonate (EMS) mainly acts on transitions in DNA molecule.

As chemical mutagens can cause a large number of desirable mutations, they are considered to be superior to physical mutagens (Athira *et al.*, 2018) [2]. In rice, there are several advantages in using chemical mutagenesis to produce mutant populations suitable for both forward and reverse genetics.

Induced mutations can be efficiently integrated with genomics, transcriptomics, proteomics and metabolomics studies to understand the phenome. However, limited information is available on their phenotypic evaluation and only small subsets of these mutants are freely available for unrestricted use (Mohapatra *et al*., 2014) [19]. With the advancement of DNA sequencing methods at present sequencing of crops has become easier. It is now possible to find the mutation responsible for the mutant phenotype of our interest. MutMap, a method that allows rapid identification of casual nucleotide changes of rice mutants by whole-genome resequencing of pooled DNA of mutant F2 progeny derived from crosses made between candidate mutants and the parental line.

The inheritance of many biological traits explored based on simple phenotying methods to reach the outcome of the established genetic models. In the case of identifying phenotypes for insect resistance in rice, there are three important components of resistance *viz*., antixenosis, antibiosis and tolerance (Painter, 1951) [24] are present. As the most important insect pests of rice, BPH demanded the attention of entomologists and breeders to develop easy and reliable screening techniques to screen a large number of germplasm and breeding materials to develop cultivars with improved resistance to BPH (Heinrichs *et al*., 1985) [10].

Thus, the present study was carried out to evaluate Nagina22 (N22) rice mutants for BPH resistance. N22 possess many traits such as drought tolerance, heat tolerance and resistance against pests and diseases, which are useful for climate-resilient agriculture. Artificial screening of N22 mutants for resistance to BPH has been carried out to identify resistant germplasm. They can be used as donors in the rice breeding program for the identification and deployment of new genes for BPH resistance (Kumar and Tiwari 2010) [16].

Materials and Methods

Mass rearing of brown planthopper (BPH), *Nilaparvata lugens*: The present experiment was conducted in controlled condition of a green-house at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. BPH was mass cultured on the susceptible rice variety Taichung Native 1 (TN1) following the method of Heinrichs *et al.* (1985) [10].

A set of 432 advanced mutant lines of N22 (Macro mutants, reported high biomass, blast resistance, drought-tolerant and mutant garden mutants) developed at Paddy Breeding station, Coimbatore was used for screening under glass-house conditions to assess the level of resistance to *N. lugens* at the seedling stage by following protray screening test (Soundararajan and Jeyaprakash 2019) [31]. These protrays are made up of polythene sheets with the size of 51 x 28cm which is commonly used for raising vegetable seedlings. They have 10 wells in lengthwise and 5 in width wise and totally 50 wells with 5.5cm diameter of each well. These wells are filled with pulverized clay soil. Each well can accommodate 15-20 seeds. The pregerminated seeds of each mutant line including standard susceptible check variety TN1, resistant check variety Ptb33 and N22 (wild) were sown. So each protray 2 wells are sown with resistant check, 2 wells with susceptible check along with 46 test entries. Three replications were maintained for each test entry. After establishment, it was thinned into 14-16 seedlings per well and maintained.

These protrays were kept in plastic trays filled with water. Seven days after sowing, the seedlings were infested with second and third instar nymphs. The plants with nymphs were gently tapped over the seedlings in such a way that approximately 6 to 8 nymphs settle on each seedling. These plastic trays were kept in wire mesh cages to prevent any escape of nymphs and to prevent entry of other insects and natural enemies.

The test entries were observed daily for the damage by the *N. lugens*. Damage grading of test mutants has been done after observing complete wilting of the seedlings in the susceptible check, TN1 by following the Standard Evaluation System (SES) for rice (IRRI, 2002) (Table 1).

Table 1: Standard Evaluation System for BPH resistance

Grade	Symptom	Rating
0	No visible damage	Immune
1	Very slight damage	Highly resistant
3	First and Second leaves of most of the plants partially turns yellowing	Resistant
5	Pronounced yellowing and stunting or about half the plants wilted or dead	Moderately resistant
7	More than half of the plants dead	Moderately susceptible
9	All plants dead	Susceptible

Selected mutants that show resistance to brown planthopper along with N22 were used for antibiosis and tolerance studies.

Antibiosis

Antibiosis is the resistance mechanism that operates after the insects have colonized and have started utilizing the plant. Various tests have been developed to determine the level of antibiosis like nymphal survival and development period and feeding rate.

Nymphal survival and development period

The seeds of selected mutants were sown in 500 ml clay pots filled with homogenized puddled soil. Thirty days old plants were infested with fresh first instar nymphs @ 10/seedling collected from the culture cages. Insects were kept confined to the plants by using Mylar cages whose open end was covered with muslin cloth. The number of nymphs that reached adulthood were counted and the percent nymphal survival was determined following the method of Heinrichs *et al.*, (1985) [10]. The number of days taken by individual nymphs to become adults were observed and the mean developmental period was calculated.

Growth index

Growth index of each test variety was calculated by dividing the nymphal survival percentage with nymphal development period recorded.

Feeding rate

Adult feeding rate was determined by quantity of honeydew

excreted using the method developed by Heinrichs *et al.*, (1985) [10]. A feeding chamber made with an inverted transparent plastic cup placed over a Whatman No. l filter paper resting on a plastic lid. Five adult females of *N. lugens* were starved for 2 h and released into the chamber through a hole at the top of the cup with thirty-day old potted plants. The hole is closed with a piece of cotton to prevent insect escape. The insects were allowed to feed for 24 h. The filter papers that have absorbed phloem-based honeydew excreted by BPH were collected and treated with 0.01% ninhydrin acetone solution. The honeydew stains appeared as violet spots due to the presence of amino acids. The area of the honeydew spots was measured by placing the filter paper over a sheet of graph paper and honeydew area is expressed as mm^2per 5 females.

Days to wilt

Days to wilt (DW) is a measure of tolerance where the damage by BPH population was estimated by counting the number of days required to kill the plants. Seeds of selected mutants were raised in pots and on the 30th day, the seedlings were caged with a cylindrical Mylar sheet cage. They were infested with second instar nymphs @ 25/plant and allowed to feed without any disturbances. The day on which the plant wilted completely was recorded.

Results and Discussion

Phenotyping

Among the 432 rice mutants screened one mutant N22-CC-DTM-893 showed resistance and which is equivalent in their resistant reaction with Ptb 33 (Resistant check) and three mutants N22-MG-145, N22-MG-491 and N22-MG-516 showed moderate resistance to BPH. The remaining all other mutants 80 categorized as moderately susceptible and 348 were categorized as susceptible and N22 (wild) show moderate susceptible reaction. This resistant mutants were taken up along with non-mutagenized N22 for further screening. Summary of the results were present in Table.2.

Table 2: Summary of BPH reaction of rice mutants

Plant damage score (Range)	No. of rice mutants	Rating
1-3	1	Resistant
3-5	3	Moderately Resistant
5-7	80	Moderately Susceptible
7-9	348	Susceptible

Understanding the phenotype is more important before embarking on its inheritance pattern to identify the gene/genes to controlling the trait. According to Soundararajan and Jeyaprakash (2019) [31] protray screening test the entries were succumbed to more susceptible to planthopper than the Standard seed box screening test (SSST) so the susceptible entries are wilted quickly in the protray screening method. Because in protray the seedlings of each entry is in group so the insects can quickly move from one susceptible plant to other plant within the genotypes and also disperse from resistant genotype to susceptible. Though protray screening method can be used as preliminary screening method when there is large number of accessions can be screened in short period than SSST and exclusion of susceptible materials to narrow down the diverse genetic material.

Whatever screening method followed, needs to provide detailed information regarding the phenotype. Among the various methods used for phenotyping to assess the level of resistance to BPH to map the genes/QTL, SSST was used by majority of the scientific community because of easiness over the other methods. Both SSST and protray methods mainly measure the plant response to nymphal feeding and preference in a free choice setup.

The major mechanisms involved in host plant resistance are antixenosis, antibiosis and tolerance (Painter, 1951) [24]. The utilization of a plant's defense mechanism is an important factor to manage crop pests. The mechanism of resistance need to be studied for ascertaining the degree of resistance among plants and it is essential for the development of durable resistant varieties. These resistant factors are heritable and they operate in a concerted manner to render plants unsuitable for insect pests. The concept of resistance mechanism could be useful to develop varieties with the most effective type of resistance against pest population (Heinrichs *et al.*, 1985) [10]. In the present study identified resistant source was further subjected to determine the mechanism of resistance and the results of these studies are discussed here under.

Nymphal survival and Development period

Significant difference among the different entries. Lower nymphal survival was observed on N22-CC-DTM-893 (26.00%) followed by Ptb33 (32.00%) while highest was observed in susceptible check (84.00%) (Table 3). Jena *et al.* (2006) [13] reported nymphal survival in highly resistant farmers varieties ranged within 10.8 to 29.2%. Results of Alagar *et al.* (2007) [1] also corroborates our finding where in the resistant genotypes had the lowest nymphal survival rate than the susceptible TN1. Similarly, Reddy *et al.* (2016) [28] also reported very low nymphal survival of BPH on some of the identified resistant sources are lower than even resistant check.

It is significant to note that, even on the most resistant lines, survival has been reported to be in the range of 30–50% (Qiu *et al.*, 2010, and He *et al.*, 2013) [27, 13] and rarely nil (Myint *et al.*, 2009). This survival rate is suggestive of lack of acute toxins as antibiotic factors in rice against BPH.

The nymphal developmental period was ranged from 10.29 to 20.43 days. Resistant check Ptb33 prolonged the developmental period of BPH nymphs (20.43 days) followed by N22-M5-BPH-893 (Table 3). But it was significantly lower in N22 (10.59 days) is on par with susceptible check TN1 (10.29 days). It is a general concept that resistant lines prolonged the developmental period and reduced the survival rate (Tingey, 1981) [32]. With respect to nymphal development, our findings support the study of Bhanu *et al.*, (2014) [4] who found that a prolonged developmental period of BPH nymphs was observed in resistant varieties. Sogawa and Pathak (1970) [30] given reasons that the prolonged nymphal period of BPH in resistant varieties was due to reduced availability or lack of required nutrients by the BPH.

The growth index differed significantly among different entries. Significantly lower growth indices were seen on Ptb33 (1.57) it is on par with N22-CC-DTM-893 (1.64) and highest was observed on TN1 (8.17) (Table 4). This index computes the adverse influence of a plant on insect survival and development rate. Several studies on planthoppers (Saxena and Okech, 1981; Du *et al.*, 2009) [29, 27] have also included both these parameters.

Table 3: Survival and developmental period of *Nilaparvata lugens* on rice entries

Entry	Nymphal survival (%) **	Nymphal duration (Days)*	Growth Index*
N22	74.00 (60.27)	10.59 (3.40)	7.00 (2.82)
N22-CC-DTM-893	26.00 (30.54)	15.80 (4.10)	1.64 (1.62)
N22-CC-MG-145	46.00 (42.62)	15.40 (4.05)	2.97 (1.98)
N22-CC-MG-491	60.00 (50.85)	11.51(3.54)	5.22 (2.49)
N22-CC-MG-516	56.00 (48.72)	13.42 (3.80)	4.17 (2.26)
Ptb33	32.00 (34.28)	20.43 (4.63)	1.57 (1.60)
TN1	84.00 (66.66)	10.29 (3.36)	8.17 (3.03)
SE(d)	5.01	0.07	0.13
CD (0.05)	10.32	0.14	0.27

* * Figures in parentheses are arc sine transformed values

* Figures in parentheses are square root transformed values

Feeding rate

Feeding rate in terms of honeydew is a useful criterion for assessing its feeding response and for grading resistance or susceptibility of the genotype. There is a significant difference in feeding rate in test entries. The lower feeding rate was observed in N22-CC-DTM-893 (58.60 mm^2) followed by Ptb33 (67.80 mm^2) while the highest in TN1 (296.00 mm^2) (Table 4). This test often complements the SSST method (Du *et al.*, 2009; He *et al.*, 2013) [27]. It is useful to measure adult female response to feeding under a no-choice setup. Alagar *et al.* (2007) [1] reported that the amount of food intake is directly proportional to the amount of honeydew excreted by BPH. Ghaffar *et al.* (2011) [8] attempted to explain why the planthopper cannot sustainably feed on resistant plants. Horgan (2009) [11] explains that associate resistance with phloem sap composition and chemistry, plant surface chemistry, volatiles and inducible responses to planthopper attack. The resistance mechanism could be a result of either diet-related primary metabolites or defence-related secondary metabolites/volatile or plant-surface characteristics or both.

Similar results were obtained by Paguia *et al.* (1980) [23] and found that more area of honeydew excretion on filter papers in susceptible variety (TN1) compared to resistant varieties (Mudgo and ASD 7). Lesser feeding of brown planthopper on resistant varieties was also reported by Lin *et al.* (2002) [17] and Udayasree and Rajanikanth (2018) [33].

Table 4: Honeydew excretion by *Nilaparvata lugens* on rice entries

Entry	Honeydew (Area in mm^2)*
N22	123.60 (11.11)
N22-CC-DTM-893	58.60 (7.68)
N22-CC-MG-145	96.20 (9.84)
N22-CC-MG-491	138.40 (11.62)
N22-CC-MG-516	110.00 (10.44)
Ptb33	67.80 (8.26)
TN1	296.00 (17.23)
SE(d)	0.81
CD (0.05)	1.67

* Figures in parentheses are square root transformed values

Tolerance mechanism against BPH in terms of days to wilt

Time taken for complete wilting of the seedlings after BPH infestation was used as a measure of tolerance. There was a significant difference among the test entries for complete wilting of mutant entries (Table 5). Resistant mutant N22-CC-DTM-893 required 35.20 days for complete wilt and which was on par with resistant check Ptb33 (31.80 days) and susceptible check TN1 take less number of days (11.00 days) for complete wilt. BPH feeding was less on resistant entries and hence the plants could withstand insect infestation. Alagar *et al.* (2007) [1] reported that resistant genotypes required more days for complete wilting of plants compared with the susceptible check TN1. Jhansi Lakshmi *et al.* (2012) [14] also reported that resistant wild rice accessions survived more than 34 days after exposure to BPH nymphs as compared to 5-6 days in susceptible check TN1 indicating the presence of a high level of tolerance mechanism. Similarly, Pati *et al.*, (2018) [26] reported highly resistant red rice accessions take more days for complete wilting of plants as like Ptb33.

Table 5: Days to wilt by *Nilaparvata lugens* in rice entries

Entry	Days to wilt*
N22	14.40 (3.91)
N22-CC-DTM-893	35.20 (6.01)
N22-CC-MG-145	19.20 (4.47)
N22-CC-MG-491	15.60 (4.06)
N22-CC-MG-516	17.20 (4.25)
Ptb33	31.80 (5.72)
TN1	11.00 (3.46)
SE(d)	0.23
CD (0.05)	0.47

* Figures in parentheses are square root transformed values

The experimental results revealed that the mutant N22-CC-DTM-893 survived after BPH infestation at the seedling stage showed a higher level of resistance to the BPH. The entry also showed substantial levels of antibiosis and tolerance effects on BPH as like resistant check.

Conclusion

Results of these studies indicated the possible use of this resistant mutant as elite genetic stock for resistance to brown planthopper (BPH), *Nilaparvata lugens*. This resource is expected to serve as a base for finding useful genes, alleles and unravelling the functional genomics of this model crop.

Conclusively, evaluation of genotypes for resistance to BPH plays a critical role in identification of new breeding material. Currently, several molecular and genomic tools are available to differentiate mutants from wild type plants. These tools can be used on mutants to identify the genes involved in resistance to BPH. A comparative analysis of up or down-regulated genes will help to identify defense-related genes and understand pathways activated in rice resistance against BPH. This information would be useful to generate rice germplasm having durable resistance to BPH.

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Reference

1. Alagar M, Suresh S, Samiyappan R, Saravanakumar D. Reaction of resistant and susceptible rice genotypes against brown planthopper (*Nilaparvata lugens*). Phytoparasitica 2007;35(4):346-56.
2. Athira S, Bakya SV, Subashini G, Muthuvijayaraghavan R, Rajeshwari S, Manonmani S, *et al*. Exploitation of induced variation for enhancing the biomass production in rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding 2018;9(4):1512-20.
3. Balakrishna B, Satyanarayana P. Genetics of brown planthopper (*Nilaparvata lugens* Stal.) resistance in elite donors of rice (*Oryza sativa* L.). The Bioscan 2013;8(4):1413-6.
4. Bhanu KV, Lakshmi VJ, Katti G, Reddy AV. Antibiosis and tolerance mechanisms of resistance in rice varieties carrying brown planthopper resistance genes. Asian Journal of Biological and Life Sciences 2014;3(2):108-13.
5. Chen M, Shelton A, Ye GY. Insect-resistant genetically modified rice in China: from research to commercialization. Annual review of entomology 2011;56:81-101.
6. Dale D. Insect pests of the rice plant–their biology and ecology. Biology and management of rice insects 1994;438:442.
7. Du B, Zhang W, Liu B, Hu J, Wei Z, Shi Z, *et al*. Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. Proceedings of the National Academy of Sciences 2009;106(52):22163-8.
8. Ghaffar MB, Pritchard J, Ford-Lloyd B. Brown planthopper (*N. lugens* Stal) feeding behaviour on rice germplasm as an indicator of resistance. PLoS One 2011;6(7):e22137.
9. He J, Liu Y, Liu Y, Jiang L, Wu H, Kang H, *et al*. High-resolution mapping of brown planthopper (BPH) resistance gene Bph27 (t) in rice (*Oryza sativa* L.). Molecular breeding 2013;31(3):549-57.
10. Heinrichs E. Genetic evaluation for insect resistance in rice: International Rice Research Institute, 1985.
11. Horgan F. Mechanisms of resistance: a major gap in understanding planthopper-rice interactions. Planthoppers: new threats to the sustainability of intensive rice production systems in Asia 2009;281:302.
12. IRRI I. Standard evaluation system for rice. International Rice Research Institute. PO Box 2002;933:1099.
13. Jena K, Jeung J, Lee J, Choi H, Brar D. High-resolution mapping of a new brown planthopper (BPH) resistance gene, Bph18 (t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 2006;112(2):288-97.
14. Jhansi Lakshmi V, Ram T, Chirutkar P, Sailaja V, editors. Mechanisms of resistance to rice planthoppers in wild rices and their accessions. International conference on Plant Health Management for Food Security, DRR Hyderabad, 2012.
15. Jiang SY, Ramachandran S. Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. International journal of biological sciences 2010;6(3):228.
16. Kumar H, Tiwari S. New Sources of Resistance Against Rice Brown Plant Hopper, *Nilaparvata Lugens* (Stal.). Indian Journal of Entomology 2010;72(3):228-32.
17. Lin Z, Dexiang G, Guren Z, Jinping Y. Behavioral responses of brown planthopper and white-backed planthopper to BPH-resistant rice varieties. Acta Phytophylacica Sinica 2002;29(2):145-52.
18. Matsumura M, Takeuchi H, Satoh M, Sanada-Morimura S, Otuka A, Watanabe T, *et al*. Current status of insecticide resistance in rice planthoppers in Asia. Planthoppers: New threats to the sustainability of intensive rice production systems in Asia 2009:233-44.
19. Mohapatra T, Robin S, Sarla N, Sheshashayee M, Singh A, Singh K, *et al*., editors. EMS induced mutants of upland rice variety Nagina22: generation and characterization. Proc Indian National Science Academy, 2014.
20. Myint KKM, Yasui H, Takagi M, Matsumura M. Virulence of long-term laboratory populations of the brown planthopper, *Nilaparvata lugens* (Stål), and white backed planthopper, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae), on rice differential varieties. Applied Entomology and Zoology 2009;44(1):149-53.
21. Narayanasamy M, Kennedy JS, Vellingiri G. Life History and Population dynamics of rice leaffolder at different temperatures 2021.
22. Normile D. Reinventing rice to feed the world. Science 2008;321(5887):330-3.
23. Paguia P, Pathak M, Heinrichs E. Honeydew excretion measurement techniques for determining differential feeding activity of biotypes of *Nilaparvata lugens* on rice varieties. Journal of Economic Entomology 1980;73(1):35-40.
24. Painter R. Insect resistance in crop plants, LWW. 1951;7(6):481.
25. Park DS, Song MY, Park SK, Lee SK, Lee JH, Song SY, *et al*. Molecular tagging of the Bph1 locus for resistance to brown planthopper (*Nilaparvata lugens* Stål) through representational difference analysis. Molecular Genetics and Genomics 2008;280(2):163-72.
26. Pati P, Jena M, Annamalai M, Behera SK, Raghu S, Bhattacharya S, *et al*. Screening of red rice accessions for resistance to brown planthopper *Nilaparvata lugens* Stal. ORYZA-An International Journal on Rice 2018;55(4):596-601.
27. Qiu Y, Guo J, Jing S, Zhu L, He G. High-resolution mapping of the brown planthopper resistance gene Bph6 in rice and characterizing its resistance in the 9311 and Nipponbare near isogenic backgrounds. Theoretical and applied genetics 2010;121(8):1601-11.
28. Reddy BN, Lakshmi VJ, Maheswari T, Ramulamma A, Katti G. Studies on antibiosis and tolerance mechanism of resistance to brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) in the selected rice entries. The Ecoscan 2016;10:269-75.
29. Saxena R, Okech S, Liquido N. Wing morphism in the brown planthopper, *Nilaparvata lugens*. International Journal of Tropical Insect Science 1981;1(4):343-8.
30. Sõgawa K, Pathak M. Mechanisms of brown planthopper resistance in Mudgo variety of rice (Hemiptera: Delphacidae). Applied entomology and zoology 1970;5(3):145-58.
31. Soundararajan R, Jeyaprakash P. Rapid screening of rice genotypes for resistance to brown planthopper, *Nilaparvata lugens* (Stal). Electronic Journal of Plant

Breeding 2019;10(1):76-82.
32. Tingey WM. The environmental control of insects using plant resistance [*Genetic diversity*]. CRC handbook of pest management in agriculture (USA). 1981.
33. Udayasree M, Rajanikanth P. Non-preference/antixenosis and antibiosis mechanism contributing to BPH resistance in certain identified elite rice genotypes. International Journal of Current Microbiology and Applied Sciences. 2018;7:1908-14.
34. Wu J, Wu C, Lei C, Baraoidan M, Bordeos A, Madamba M. Ramos-Pamplona M, *et al*. Chemical and irradiation induced mutants of indica rice IR64 for forward and reverse genetics. Plant Molecular Biology 2005;59(1):85.